YopM Puts Caspase-1 on Ice

Ine Jørgensen[1] and Edward A. Miao[1,*]
[1]Department of Microbiology and Immunology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
*Correspondence: emiao@med.unc.edu
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Caspase-1-mediated detection of pathogens is a potent arm of the innate immune system. LaRock and Cookson (2012) show that the *Yersinia* type III secretion effector, YopM, directly inhibits caspase-1.

The innate immune system detects pathogens through cytosolic inflammasomes, including the Nod-like receptors (NLRs) NLRP3, NLRC4, and NLRP1b. These signaling platforms recruit the adaptor protein ASC into a single focus. The ASC focus subsequently recruits the enzyme caspase-1, which autoprocesses and promotes the secretion of the proinflammatory cytokines IL-1β and IL-18 that initiate immune responses. Underscoring the importance of caspase-1 in the innate immune response, mice deficient in caspase-1 fail to control many pathogenic infections. Many viruses have developed direct mechanisms to suppress either caspase-1 or IL-1β, delaying the onset of the innate immune response. For example, poxviruses encode direct inhibitors of caspase-1, such as CrmA, as well as analogs of the mammalian pyrin-only proteins that likely prevent ASC oligomerization (Taxman et al., 2010). Further, Kaposi's sarcoma-associated herpesvirus (KSHV) encodes a NLR analog that inhibits inflammasome oligomerization (Gregory et al., 2011). Macrophages detect *Salmonella* Typhimurium though the NLRC4 inflammasome when the *S.* Typhimurium type III secretion system (T3SS) inadvertently translocates flagellin or T3SS rod proteins into the host cell cytosol. *S.* Typhimurium indirectly evades this detection by repressing flagellin expression and using an alternate NLRC4-evading T3SS rod proteins (Miao and Rajan, 2011). However, bacterial virulence proteins that directly inhibit inflammasome pathways have not been identified. *Yersinia* drastically modulates the host environment and suppresses immune signaling by delivering effectors (Yops) into the host cytosol via its T3SS. In this issue of *Cell Host & Microbe*, LaRock and Cookson elegantly demonstrate that the *Yersinia* T3SS effector YopM directly inhibits caspase-1 and that this inhibition is essential for *Yersinia pseudotuberculosis* virulence in vivo (LaRock and Cookson, 2012).

In their report, wild-type *Y. pseudotuberculosis* failed to activate caspase-1, whereas caspase-1 was robustly activated in response to a strain deficient in all known T3SS effector Yop genes (*Yersinia*Δ lacking YopJOEHKM). Remarkably, LaRock and Cookson found that evasion of caspase-1 was entirely attributed to *yopM*. This was proven using a strain deficient only in *yopM*, complementing Δ*yopJOEHKM* with plasmid borne *yopM*, and retrovirally transducing macrophages with y*opM* in the absence of bacterial infection. It has long been known that the Δ*yopM* mutant is highly attenuated, although its function in vivo has remained elusive. LaRock and Cookson nail down the importance of YopM-mediated caspase-1 inhibition by demonstrating that Δ*yopM* becomes fully virulent in caspase-1-deficient mice (LaRock and Cookson, 2012).

Significantly, LaRock and Cookson provide a mechanism by which a bacterial virulence factor inhibits caspase-1 activity (Figure 1). Several viruses inhibit caspase-1 by expressing proteins that mimic the caspase-1 binding and cleavage motif and thus act as pseudosubstrates that block the caspase-1 catalytic site. By aligning YopM to known caspase-1 substrates and inhibitors, the authors identified a caspase-1-binding motif. The critical aspartic acid residue within this motif was required for direct YopM binding to activated caspase-1. Interestingly, the YopM C terminus directs nuclear localization, and this domain is distinct from the caspase-1-binding site (Benabdillah et al., 2004). LaRock and Cookson's data suggest an intriguing model in which YopM carts caspase-1 into the nucleus, making it unavailable for recruitment to the cytosolic ASC focus. Indeed, the NLRP3-ASC focus still forms, yet YopM prevents caspase-1 accumulation in this focus.

Several groups previously investigated inflammasome detection of *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*, focusing on YopE, YopJ, and YopK. These reports must now be reinterpreted in light of YopM suppression. It is worth noting that the use of different cell lines (primary versus immortalized), macrophage priming conditions, and *Yersinia* strains may explain the differences in the relative contribution of different Yops in suppressing caspase-1 activation. For example, Schotte et al. (2004) reported that *Y. enterocolitica* Δ*yopHOPEMT* triggered IL-1β secretion and suggested that YopE, but not YopM, was the critical effector blocking this response. However, they examined only a single macrophage cell line in the absence of priming, with complementary studies in caspase-1-overexpressing cell lines. Does caspase-1 activation in these cell lines accurately reflect detection pathways occurring in primary macrophages and in vivo?

Later, using a plasmid-cured *Y. pseudotuberculosis* strain containing the *Y. pestis* virulence plasmid deleted for all known Yop effectors (*yopM*, *yopT*, *yopK*, *ylpA*, *yopE*, *yadA*, *ypkA*, *yopJ*, *yopH*) and several other open reading frames, YopK was implicated in decreasing caspase-1 activation and IL-1β secretion (Brodsky et al., 2010). YopK interacts with the YopBD translocon, and in the absence of all known Yops, adding back YopK results in decreased caspase-1 activation. Pore-forming toxins are detected through NLRP3. Thus, in the absence of YopK, does the YopBD translocon pore become dysregulated, permitting

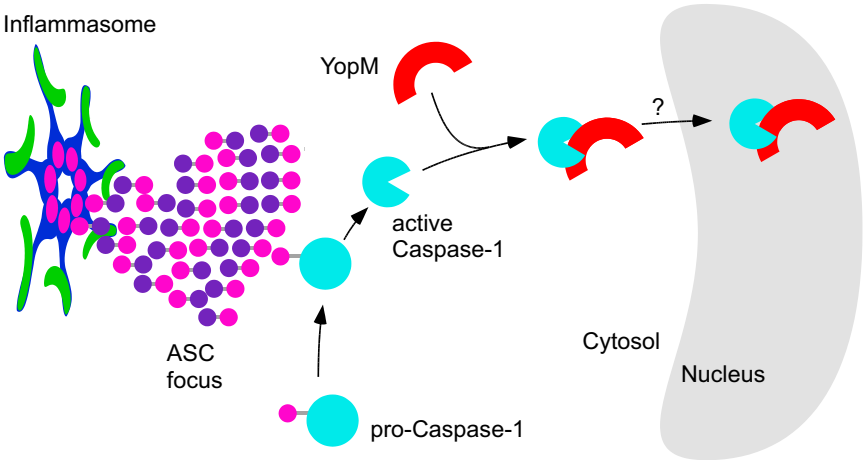

Figure 1. YopM Inhibits Caspase-1

Yersinia T3SS activity is detected by cytosolic inflammasomes by unknown mechanisms. Oligomerized NLR inflammasomes recruit the adaptor protein ASC through homotypic interactions between CARD (pink) or pyrin (purple) domains. ASC is composed of a CARD and a pyrin domain and subsequently collects the entire complement of cellular ASC into a single focus. The ASC-CARD domains then recruit pro-caspase-1 via its CARD domain, resulting in its autoproteolytic processing and activation. YopM contains a pseudosubstrate binding site that binds and retains activated caspase-1. Because YopM contains a nuclear localization signal, sequestration of activated caspase-1 in the nucleus may be a key mechanism by which YopM separates caspase-1 from its intended targets.

potassium efflux from the cell and subsequent NLRP3 activation? Interestingly, *yopK* mutants have an increased rate of translocation of other effectors, including YopM (Dewoody et al., 2011). Are increased amounts of T3SS rod protein and flagellin aberrantly injected into the cell to be detected though NLRC4? Perhaps in the absence of YopK, rapid detection kinetics though multiple pathways overcome the ability of YopM to block caspase-1.

In stark contrast to YopM/YopK suppressing inflammasome activation, YopJ (a MAPK and IKKβ inhibitor also known as YopP in *Y. enterocolitica*) induces NLRP3-dependent caspase-1 activation and IL-1β secretion in the absence of priming (Viboud and Bliska, 2005). Interestingly, YopJ/YopP isoforms that are more catalytically active are more readily detected, with IL-1β secretion at 8–24 hr postinfection (Zheng et al., 2012). This is quite delayed compared to typical inflammasome agonists such as *S.* Typhimurium, ATP, or Alum. Are these delayed kinetics due in part to YopM? To fully understand the ability of inflammasomes to detect the catalytic activity of YopJ, experiments comparing wild-type, Δ*yopM*, Δ*yopJ*, and Δ*yopMJ* in primed as well as unprimed macrophages would be informative.

Although LaRock and Cookson show that caspase-1 inhibition is the primary function of YopM, it may have other functions. YopM is polymorphic, and the number of leucine-rich repeats, which mediate protein-protein interactions, varies between strains. One could imagine that by adding more leucine-rich repeats, a greater surface area for binding host proteins could be created. YopM binds two host serine/threonine kinases (RSK1 and PKN2) (Viboud and Bliska, 2005). Does YopM bind caspase-1 and RSK1/PKN2 at the same time and sequester all three in the nucleus? Thus, it is possible that YopM plays different functions in different *Yersinia* species and/or targeted cell types.

In summary, LaRock and Cookson provide convincing data that the T3SS effector YopM promotes *Yersinia* virulence by inhibiting caspase-1 activation, presumably permitting early replication in the absence of an inflammatory response. This story exemplifies the complex interplay between innate immune responses and bacterial evasion strategies. Whereas delivery of effectors by the T3SS into the host cytosol is a highly efficient mechanism by which pathogens modify the host environment to their advantage, it also provides a smorgasbord of bacterial ligands and cellular perturbations that can be detected by cytosolic sensors. In *Yersinia*, YopM counters this inherent weakness in the system.

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